

Appl. No. : 10/090,038
Filed : February 27, 2002

REMARKS

This Amendment and Response is submitted in response to the Final Office Action mailed October 20, 2004. Claim 1 has been amended to include the term “synergistically” with reference to the effective amount of chromium complex and biotin. Support for the amendment can be found throughout the specification and in the original claims as filed. For example, support for the amendment to Claim 1 can be found in Figures 2 and 14 and pages 15 and 25 of the Specification. No new matter has been added. Claims 1-20 and 23-54 are pending and presented for examination. Reconsideration and withdrawal of the present rejections in view of the amendments and comments presented herein are respectfully requested.

Claims 1, 4-6, 8, 10-13, 17 and 19 are novel under 35 U.S.C. §102(b) in view of Rath (U.S. Patent No. 6,693,129)

Claims 1, 4-6, 8, 10-13, 17, and 19 were rejected under 35 U.S.C. §102(b) as anticipated by or, in the alternative, under 35 U.S.C. §103(a) as obvious over Rath (U.S. Patent No. 6,693,129). According to the PTO, Rath expressly discloses a method of treating high LDL and high triglycerides by administering a composition containing biotin and chromium glycinate, which allegedly falls within the scope of the claims of the present application. Under 35 U.S.C. § 102(b), a claim is anticipated only if the reference reads on the claim. M.P.E.P. §2131. Each element as set forth in the claims is not found in the Rath patent. Accordingly, Applicants respectfully submit that Claims 1, 4-6, 8, 10-13, 17, and 19, are not anticipated by the Rath patent and request withdrawal of the PTO’s rejection under 35 U.S.C. §102(b).

A review of the Rath patent reveals that it discloses compositions and methods for lowering plasma Lp(a) levels in humans. As Table 1 beginning on Column 6, line 45 of the Rath patent makes clear, the composition for accomplishing the lowering of plasma Lp(a) levels includes no less than thirty-five biochemical compounds in addition to biotin and chromium glycinate. Notably, the Rath reference requires numerous active ingredients to achieve the claimed beneficial health effects. In contrast, Claim 1 and the claims depending therefrom recite a method for treating dyslipidemia *consisting essentially of* administering an effective dose of two bioactive ingredients, a chromium complex and biotin.

The transitional phrase “consisting essentially of” has a well-established meaning, which limits the scope of a claim by excluding additional materials or steps that materially affect the basic and novel characteristics of the invention. See Atlas Pander Co. v. E.I. duPont de Nemours

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& Co., 750 F.2d 1569, 224 USPQ 408 (Fed Cir. 1998). M.P.E.P. §2111.03. Several cases decided by the courts have clarified the meaning of “materially affect” as it relates to the transitional phrase “consisting essentially of.” Courts have repeatedly found that the transitional phrase “consisting essentially of” excludes all ingredients that impact the basic and novel characteristic(s) of the invention. Clearly, all active ingredients that affect the basic and novel characteristics of the invention are excluded from the phrase “consisting essentially of.” This is demonstrated in the tobacco wrapper case. The district court of New Jersey affirmed the findings of the Board of Patent Interference Examiners and held that “consisting essentially of” excluded materials that had a complimentary or beneficial effect on tobacco paper. American Machine & Foundry Co. v. Liggett & Myers Tobacco Co., 172 F.Supp. 12 (D.C.N.J. 1959). Similarly, in PPG Industries v. Guardian Industries Corp., the Federal Circuit excluded all materials that affect spectral properties of glass, holding that any ingredient which caused measurable changes in the transmittance or dominant wavelength of the glass would necessarily be excluded from a “consisting essentially of” claim.

Claim 1 and the claims depending therefrom recite a composition consisting essentially of two specific bioactive ingredients, and therefore exclude other components that materially affect the basic and novel characteristics of the invention. In addition to biotin and chromium glycinate, the Rath patent recites additional components that materially affect the treatment of dyslipidemia. For example, the Rath composition comprises L-arginine for lowering the risk factors for cardiovascular diseases including dyslipidemia. L-arginine has been reported to normalize glycemia and reduce hyperlipidemia (see Exhibit A, submitted herewith). Thus, arginine administration would *materially affect* lipid levels in an individual. Similarly, L-carnitine is known to support fat metabolism to improve cardiovascular health. As reported in Exhibit B (submitted herewith), L-carnitine aids in the clearance of triglycerides and fatty acids from the blood stream, thereby *materially affecting* lipid metabolism in an individual. In addition, the Rath composition includes folic acid, another bioactive ingredient associated with the reduction of LDL (the so-called “bad cholesterol”). See Exhibit C, submitted herewith. These additional components, on the face of the Rath patent, substantially influence the pharmacological effect of the composition and efficacy of the treatment of dyslipidemia. Importantly, these components are absent from Claim 1 and the claims depending therefrom of the present application.

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Because the present claims are limited to compositions consisting essentially of specific bioactive ingredients, namely biotin and a chromium complex, and because the Rath patent teaches compositions comprising additional components that influence the pharmacological effect of the compositions, the Rath reference fails to teach every limitation of the claims. Accordingly, the Rath patent does not anticipate Claims 1, 4-6, 8, 10-13, 17 and 19.

Claims 1, 4-6, 8, 10-13, 17 and 19 are non-obvious under 35 U.S.C. §103(a) over Rath (U.S. Patent No. 6,693,129)

Claims 1, 4-6, 8, 10-13, 17 and 19 were likewise rejected under 35 U.S.C. §103(a) as being obvious in view of Rath. According to the PTO, the claimed invention is rendered obvious within the meaning of 35 U.S.C. 103, because the prior art allegedly discloses products and uses that contain “the same exact ingredients/components as that of the claimed invention.” See page 2 of the Final Office Action. Applicants assert that the PTO has failed to give sufficient weight to each limitation of the claims, in particular, the use of the transitional phrase “consisting essentially of”, when evaluating whether the claimed invention is obvious in view of the prior art. As will be discussed in greater detail below, Applicants submit that Claims 1, 4-6, 8, 10-13, 17 and 19 are non-obvious in view of the Rath patent and respectfully request withdrawal of the rejection for the following reasons.

In order to articulate a *prima facie* case for obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine the reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art references must teach or suggest all the claim limitations. See M.P.E.P. § 2143. Applicant submits that the contrasts between the present invention and the Rath patent are significant and the reference is not sufficient to support a *prima facie* case of obviousness.

There is no motivation to modify the reference

The Examiner has pointed to no motivation to modify the Rath patent to arrive at the claimed method of treating dyslipidemia. The Rath patent is directed to a multi-component formulation for the treatment of cardiovascular disorders. Specifically, the formulation described in the Rath patent includes over thirty-five ingredients. The PTO opines that it would have been obvious to omit thirty-five other bioactive ingredients to arrive at a composition consisting essentially of chromium and biotin for treating dyslipidemia.

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The mere fact that the teachings of Rath patent might be modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. See In re Mills, 16 U.S.P.Q.2d 1430 (Fed Cir. 1990); See, also M.P.E.P. §2143.01. The Rath patent is silent with respect to the advantages of selecting only two of the almost forty components to treat dyslipidemia as is presently claimed. There is absolutely no teaching or indication in the reference to remove the thirty-five other possible bioactive components from a pharmaceutical composition to arrive at a method of treating dyslipidemia with only two bioactive ingredients, namely chromium and biotin, as is presently claimed. Further, there is great benefit in being able to narrow down the number of active ingredients one must use.

As stated above, Claim 1 and the claims depending therefrom are directed to a method consisting essentially of administering a chromium complex and biotin. In In re Fine, the Federal Circuit made clear that “[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention.” 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988). Instead, there must be some reason, suggestion, or motivation found in the cited references whereby a person of ordinary skill in the art would make the combination and that knowledge cannot come from the applicant’s disclosed invention. See In re Oetiker, 24 U.S.P.Q.2d 1443 (Fed. Cir. 1992). Absent impermissible hindsight, it would not have been obvious to strip away the multitude of efficacious bioactive components disclosed in Rath, select only two bioactive components, and arrive at a method of treating dyslipidemia consisting essentially of administering a chromium complex and biotin. Accordingly, Claims 1, 4-6, 8, 10-13, 17, and 19 are not obvious in view of the Rath patent.

No reasonable expectation of success in modifying or combining the cited reference

Furthermore, a *prima facie* case for obviousness is established only when the PTO provides a reference or references that would lead one of ordinary skill in the art to believe that he or she would have a reasonable expectation of success in practicing the claimed invention in view of the cited art. See In re Merck & Co., Inc., 231 U.S.P.Q. 375 (Fed. Cir. 1986); M.P.E.P. §2143.02. Applicant respectfully submits that it is not reasonable to expect that two ingredients selected from the numerous possible bioactive ingredients disclosed in Rath, would act complementarily to treat dyslipidemia as claimed in the present invention.

Applicant further submits that there is no reason to believe that the teachings of Rath describe a method of treating dyslipidemia consisting essentially of administering a chromium

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complex and biotin as described in Claim 1 and the claims depending therefrom of the present invention. Due to the unpredictable synergistic result of the combination of chromium and biotin as claimed in the present invention, it would not have been obvious to one of skill in the art to derive a method of treating dyslipidemia consisting essentially of administering a chromium complex and biotin from the multi-component formulation described in Rath. For these reasons, Claims 1, 4-6, 8, 10-13, 17, and 19 are non-obvious in view of Rath.

The references do not teach or suggest all of the claim limitations

Finally, in order to establish a case for obviousness, the Examiner must cite prior art that teaches or suggests all the claim limitations. See M.P.E.P. § 2143.03. For the same reasons set forth in the response to the § 102 rejection, the Rath patent fails to teach or suggest all of the claim limitations. As outlined above, Claims 1, 4-6, 8, 10-13, 17, and 19 relate to a method for treating dyslipidemia consisting essentially of administering a chromium complex and biotin. As noted above, the “consisting essentially of” transitional phrase limits the scope of a claim to the specified materials or steps and excludes materials or steps that materially affect the basic and novel characteristics of the invention. Because the Rath patent recites and claims a composition comprising far more than a chromium complex and biotin, it does not read on the pending claims. The reference therefore does not teach each and every limitation of claims 1, 4-6, 8, 10-13, 17 and 19, and therefore fails to establish *prima facie* obviousness.

In view of the deficiencies discussed above, the Rath reference is not sufficient to support a *prima facie* case of obviousness. Therefore, Applicants request withdrawal of this rejection.

Claims 1-20 and 23-54 are non-obvious under 35 U.S.C. §103(a) in view of McCarty (U.S. Patent No. 5,789,401) or McCarty (U.S. Patent No. 5,929,066), in further view of de la harpe et al. (U.S. Patent No. 5,948,772) and Brand-Miller

Claims 1-20 and 23-54 were rejected under 35 U.S.C. §103(a) as being unpatentable over McCarty et al. (U.S. Pat. No. 5,789,401) or McCarty (U.S. Pat. No. 5,929,066) in view of de La Harpe (U.S. Pat. No. 5,948,772), Jensen (U.S. Pat. No. 5,194,615) and Brand-Miller (*Am. J. Clin. Nutr.* 59(suppl):747S-752S, 1994). As detailed above, three basic criteria must be met in order to establish a *prima facie* case of obviousness. M.P.E.P. §2142. If the prior art fails to meet one or more of the criteria, a *prima facie* case of obviousness cannot be asserted. The prior art references have been extensively characterized in previous Office Actions and Amendment and Responses to Office Actions. For the sake of brevity, Applicants will not duplicate prior

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arguments or repeat previous characterizations of the prior art. However, Applicants continue to maintain that the PTO has failed to establish a *prima facie* case of obviousness and request withdrawal of the claim rejections under 35 U.S.C. §103(a) for the reasons of record and for the reasons articulated below.

The PTO states that the rejection of Claims 1-20 and 23-54 as obvious under 35 U.S.C. §103(a) is based on a combination of references which allegedly teach or suggest the claimed invention. The PTO reiterates its position that Applicants have impermissibly attacked references individually rather than considering what the combined teachings of the references would have suggested to one of ordinary skill in the art. While Applicants agree with the PTO's position that there is no requirement that the claimed invention be expressly suggested in any one or all of the references, Applicants maintain that there must be disclosure or a suggestion for each idea linking the methods of the present claims to their intended purposes, with a reasonable expectation of success somewhere in the combined disclosure of the references. Applicants assert that the cited art fails to provide the requisite motivation to practice the claimed invention.

Claims 1-20 of the present invention are directed to a method of treating dyslipidemia *consisting essentially of* administering chromium and biotin. Claim 23 and the claims depending therefrom relate to a method of reducing the glycemic index of food by administering chromium and biotin. Claim 31 relates to a method for lowering post-prandial hyperglycemia via the administration of chromium and biotin. The present invention is based, in part, on the surprising discovery that the co-administration of chromium and biotin can facilitate the treatment and recovery of individuals suffering from a variety of medical conditions including dyslipidemia and hypercholesterolemia caused or exacerbated by insulin insensitivity or post-prandial hyperglycemia. Applicants have discovered that if you take a chromium complex in combination with biotin, one can prevent the elevation of glucose levels in response to food. As stated above, the use of the transitional phrase "*consisting essentially of*" in Claim 1 limits the claim by excluding additional materials or steps that materially affect the basic and novel characteristics of the invention. None of the references cited by the Examiner teach or suggest a composition or method *consisting essentially of* merely two bioactive ingredients, namely chromium and biotin. Additionally, all of the cited references are completely silent with respect to methods or compositions for preventing the elevation of glucose levels in response to food, *i.e.* a method of

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treating post-prandial hyperglycemia. Furthermore, none of the references disclose or suggest a mechanism by which the glycemic index of food can be lowered as is presently claimed.

Applicants submit that there is no support in the prior art for the PTO's inference that a treatment for ineffective insulin and compromised glucose metabolism would subsequently provide a treatment for hypercholesterolemia in diabetes as well. No mechanism for the activity of biotin is suggested, disclosed or supported by the combined disclosure of the prior art.

Turning to the specifics of the PTO's argument, the PTO asserts that the de La Harpe patent ('772) clearly discloses that hypercholesterolemia is present in diabetes. The PTO then opines that since diabetics suffer from ineffective insulin and compromised glucose metabolism and since hypercholesterolemia is present in diabetics, that one with skill in the art would expect that by administering biotin, a substance "known to make insulin more effective" according to the PTO, hypercholesterolemia can be treated. However, the PTO is erroneously drawing a causal connection between hypercholesterolemia and diabetes, a connection which is neither taught nor suggested by the prior art references. Specifically, the PTO has mistakenly concluded that since diabetics suffer from ineffective insulin and compromised glucose metabolism and that hypercholesterolemia is often present in diabetics, it follows that the ineffective insulin and compromised glucose metabolism are the cause of hypercholesterolemia in diabetics and that by treating ineffective insulin and compromised glucose metabolism, hypercholesterolemia will also be treated.

The PTO has seemingly ignored the fact that there is simply no factual support in the prior art references for such conclusions. Applicants reiterate that the coincidence of multiple symptoms or syndromes indicative of disease in a patient population is not evidence of a causal relationship between those symptoms or syndromes. The symptoms or syndromes may be unrelated or they may each be an effect of an additional condition. None of the references, alone or combined, suggest a causal relationship in which hypercholesterolemia is a consequence of ineffective insulin and compromised glucose metabolism.

There is also no disclosure or suggestion in the references as to a mechanism by which biotin affects glucose tolerance. According to the PTO, it is known from the disclosures of the prior art that biotin acts by making insulin more effective. However, Applicants submit that the disclosure of the prior art references makes no such claim and actually teaches away from the PTO's conclusion. Any judgment on obviousness must only be based on knowledge which was

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within the level of ordinary skill in the art at the time the claimed invention was made. The mere fact that the reference teachings might be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. See In re Mills, 16 U.S.P.Q.2d 1430 (Fed Cir. 1990); See, also M.P.E.P. §2143.01. Applicants submit that any evidence or suggestion of the effectiveness of biotin in altering serum lipid levels in any circumstance or as part of any composition is found exclusively in the disclosure of the present application. In In re Fine, the Federal Circuit made clear that “[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention.” 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988). Instead, there must be some reason, suggestion, or motivation found in the cited references whereby a person of ordinary skill in the art would make the combination and that knowledge cannot come from the applicant’s disclosed invention. See In re Oetiker, 24 U.S.P.Q.2d 1443 (Fed. Cir. 1992). Absent impermissible hindsight, it would not have been obvious to a skilled artisan that biotin would be effective in altering serum lipid levels. As there is no suggestion that biotin supplementation would affect lipid levels in any way in the cited prior art, the use of biotin to affect lipid levels is not obvious.

The PTO alleges that Applicants have provided no evidence that the use of the phrase “consisting essentially of” excludes one or more of the components in the Rath patent. Applicants assert that they have fully articulated why the use of the phrase “consisting essentially of” excludes all bioactive ingredients enumerated in the Rath patent which materially affect the basic and novel characteristic of the claimed invention. The citation of Rath, therefore, adds nothing to the PTO’s assertion that the present claims are obvious in view of the prior art and cannot support a case for obviousness. As detailed above, the Rath patent does not disclose or suggest the claimed invention as the Rath patent fails to teach each and every limitation of the claims as required under 35 U.S.C. §103(a). Further, Applicants’ characterization of this claim term in this file history is the type of evidence that is relied on by courts in interpreting the claim. The PTO is justified in relying on the Applicants’ interpretation of the claim limitation, and Applicants are entitled to define or ascribe meaning to claim terms.

As the combined disclosure of the prior art references fails to meet any of the criteria for a *prima facie* case of obviousness, Applicants respectfully request the withdrawal of the rejection of Claims 1-20 and 23-27 under 35 U.S.C. §103(a) as being unpatentable in view of the cited prior art.

The synergistic effects of chromium and biotin on serum lipid levels is unexpected

The PTO restates in the pending Office Action that the evidence of synergy is not commensurate in scope with the breadth of the claims because “only specific amounts are tested and only glucose uptake and HDL-change are shown.” According to the PTO, the McCarty patents disclose the synergistic effects of administering a composition of chromium and biotin, thus any synergy seen in the administration of chromium and biotin would be expected. Applicants respectfully disagree.

Figures 2 and 14 present evidence of the synergy of chromium and biotin on changes in HDL levels and glucose uptake. In each experiment, a variety of chromium and biotin amounts are administered alone or in combination. The figures show that the administration of a composition of chromium and biotin together has a greater than additive effect on glucose uptake and on changes in HDL levels in laboratory rats.

Contrary to the position taken by the PTO, the specific amounts of biotin and chromium tested are more than sufficient to show the synergistic effects. One with skill in the art would expect to see synergistic effects with the administration of other amounts of chromium and biotin found in the ranges given in the claims. Additionally, one with skill in the art would appreciate that the absolute levels of various blood lipids is not as important as the ratios between the concentrations of the lipids when evaluating a patient’s risk for disease due to dyslipidemia. Hence, a rise in HDL levels would indicate reduced risk for disease and would be evidence of a

The prior art collectively contains no support or suggestion of a role for biotin in the treatment of dyslipidemia and does not disclose, suggest or teach that a composition of chromium and biotin administered together would have any effect on serum lipid profiles beyond those seen with the administration of chromium by itself, let alone *synergistic* effects on changes in HDL levels. Hence, the combined disclosure of the prior art fails to meet any of the criteria for a *prima facie* case for obviousness.

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CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully submit that the present application is in condition for allowance. Nevertheless, the PTO is invited to contact the undersigned at the telephone number appearing below to discuss any remaining issues. Any claim amendments which are not specifically discussed in the above remarks are made in order to improve the clarity of claim language, to correct grammatical mistakes or ambiguities, and to otherwise improve the capacity of the claims to particularly and distinctly point out the invention to those of skill in the art.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: _____

12/16/04

By _____



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Regulation of hyperglycemia and dyslipidemia by exogenous L-arginine in diabetic rats.

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The effect of L-arginine on the pattern of lipids and lipoproteins in normal and diabetic rats was studied. Three groups of 48 rats were studied during 12 days and compared with a control group (Group I, n = 5). Group I consisted of normal rats not treated with L-arginine. Group II. Normal rats treated with 10 mM L-arginine (i.p.). Group III. Diabetic rats (alloxan 120 mg/kg, i.p.) not treated (diabetic control). Group IV. Diabetic rats treated with 10 mM L-arginine (i.p.). The rats of each group were divided in subgroups of four each. Rats were anesthetized and blood was taken from aorta to determine glucose, triglycerides, cholesterol, total lipids, and low (LDL) and high density lipoproteins (HDL) and their corresponding apoproteins (Apo A-I and Apo B-100). We observed that the alloxan concentration used in this study reproduces the clinical manifestations of disease including hyperglycemia (from 132.5 +/- 7.6 to 544.3 +/- 16.9 mg/dL) in 96 h. As a consequence the levels of triglycerides, cholesterol, total lipids, and LDL and its apoprotein Apo B-100 were increased, whereas HDL and its apoprotein Apo A-I were diminished. The L-arginine injection tends to normalize the glycemia from 24 h; similarly, hyperlipidemia (triglycerides from 924.7 +/- 220.1 to 68.5 +/- 8.4 mg/dL, cholesterol from 107.7 +/- 0.6 to 64.5 +/- 4.2 mg/dL, LDL from 24.2 +/- 2.5 to 8.0 +/- 2.9 mg/dL) was also diminished. These results suggest that the beneficial effect of L-arginine administration on serum glucose values and lipid levels in diabetic rats can be mediated by polyamine formation, although the effect of L-arginine on insulin release as observed by other authors is not discarded.

Major Subject Heading(s)	Minor Subject Heading(s)	CAS Registry / EC Numbers
<ul style="list-style-type: none"> Arginine [metabolism] Hyperglycemia Hyperlipidemia 	<ul style="list-style-type: none"> Animals Aorta [metabolism] Apolipoprotein A-I [metabolism] Apolipoproteins B [metabolism] Blood Glucose [metabolism] Body Weight Cholesterol [metabolism] Diabetes Mellitus, Experimental Glucose [metabolism] Lipoproteins, HDL [metabolism] Lipoproteins, LDL [metabolism] Rats, Sprague-Dawley Rats Time Factors Triglycerides [metabolism] 	<ul style="list-style-type: none"> 0 (Apolipoprotein A-I) 0 (Apolipoproteins B) 0 (Blood Glucose) 0 (Lipoproteins, HDL) 0 (Lipoproteins, LDL) 0 (Triglycerides) 0 (apolipoprotein B-100) 50-99-7 (Glucose) 57-88-5 (Cholesterol) 74-79-3 (Arginine)

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COCHRAN FOUNDATION OF MEDICAL RESEARCH *Specializing in Cardiovascular and Autoimmune Diseases · Clinical Test Trials Operating in United States and India · Eight Medical Doctors and Biochemist on Staff with over 200 years of medical training and experience*

- **L-CARNITINE** Report up-date on June 8, 1997

L-Carnitine supports fat metabolism for cardiovascular health. L-Carnitine transports long-chain fatty acids into the mitochondria of each cell. The fats are then broken down into energy, which is used to fuel the cardiovascular and Muscular Systems of the body.

When insufficient levels of L-Carnitine are present, then too few fatty acids are transported into the cell. Instead of being effectively utilized as fuel, the fat builds up in the bloodstream and as a result, lethargy, muscle weakness, and fatigue will happen. Long term cardiovascular health and maintenance is sacrificed in the process.

L-Carnitine is found naturally in red meat (So is Coenzyme Q-10) so the supplementation for vegetarians is of the uppermost importance. Especially since as we age the level of this amino acid drops throughout the aging process. In addition, as the body ages, it becomes less efficient at metabolizing nutrients, so that the elderly will benefit greatly from L-Carnitine supplements.

By enabling the body to operate more efficiently by utilizing fat for energy, L-Carnitine enhances Energy, Stamina and metabolic rate levels. Clinical studies have shown an increase in exercise and cardiovascular tolerance and a decrease in fatigue in cardiovascular disease patients who took supplemental L-Carnitine. By ensuring adequate utilization of fatty acids to fuel the heart and other essential organs, L-Carnitine is able to help combat fatigue, enhance the body's energy levels and help build lean muscle mass and increase stamina and the metabolic rate of the body.

Carnitine is important in the regulatory effect upon fat metabolism in the heart and skeletal muscles. Under our medical trials it has been shown to stimulate fat metabolism and it assists in the clearance of triglycerides and fatty acids from the blood stream. In human metabolism, the amino acid transport system is utilized in the transfer of fatty acids across the cell membrane and on to the mitochondria. It is here at the mitochondria level; once the fatty acids are finally delivered that they can be used as an efficient source of fuel for generating energy on a cellular level for the body. Carnitine is not a vitamin but is an amino acid, and is found only in animal muscle tissue and organs. Carnitine can't be source from vegetable or fruit diets. The highest concentration level of Carnitine found in the human body is in one's internal organs and skeletal muscle groups.

We have found that there is a great different between men and woman for the internal need for this amino acid. Men have and need to have a much a higher blood level of Carnitine then women. This is because the highest level of Carnitine is found in the epididymis of the testes in males. Carnitine is necessary for energy metabolism with in the sperm for proper mobility, to strengthen and enhance the sperm metabolic rate for fertilization. In humans or mammals that have low to very low Carnitine blood level, sperm just doesn't have the proper strength levels to supply them with the required energy to complete their course to the female egg in waiting.

Our Medical trials are currently underway and we have experienced some very striking results with regards to both cardiovascular diseases and Fertility. Additional information will be added when our trial data is reviewed and complied.

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Last modified: February 22, 2001



Does Oral Folic Acid Lower Total Homocysteine Levels and Improve Endothelial Function in Children With Chronic Renal Failure?

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A. Donald, AVT; G. Oakley, RGN, MSc; Z. Varghese, PhD, FRCPath;
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Background—Accelerated vascular disease is common in chronic renal failure (CRF) and accounts for significant mortality and morbidity. Elevated homocysteine levels may contribute by an effect on endothelial function.

Methods and Results—We performed a double-blind placebo-controlled randomized crossover trial of folic acid at 5 mg/m² in 25 normotensive children 12±3 (7 to 17) years of age with CRF (glomerular filtration rate 26.8±13.2 mL/min per 1.73 m²) of noninflammatory etiology. Each subject underwent two 8-week periods of folic acid and placebo separated by an 8-week washout period. The effect of folic acid on homocysteine levels, LDL oxidation, and both endothelial-dependent and -independent vascular function were measured. After oral folic acid, serum folate levels rose from 11.7±4.25 to 635±519 μg/L ($P=0.001$), red cell folate levels rose from 364±195 to 2891±2623 μg/L ($P<0.001$), and total homocysteine levels fell from 10.28±4.16 to 8.62±2.32 μmol/L ($P=0.03$). In addition, there was a significant improvement in flow-mediated dilatation (FMD) (endothelial-dependent dilatation) from 7.21±2.8% to 8.47±3.01% ($P=0.036$) with no change in response to glyceryl trinitrate (endothelial-independent dilatation). There was no significant change in FMD or glyceryl trinitrate during the placebo phase. There was, however, no significant difference in final FMD after placebo or folic acid. Lag times for LDL oxidation were prolonged during the treatment phase (58.4±18.7 to 68.1±25.9 minutes, $P=0.01$).

Conclusion—Folic acid supplementation in children with CRF may improve endothelial function with an increased resistance of LDL to oxidation. (*Circulation*. 2002;105:1810-1815.)

Key Words: homocysteine ■ folic acid ■ renal failure ■ endothelium

Premature atherosclerosis is a major cause of morbidity and mortality in adults with chronic renal failure (CRF). This may be due not only to the increased incidence of classic risk factors such as glucose intolerance, hypertension, and dyslipidemia but also to a direct adverse effect of CRF.¹

We have demonstrated endothelial dysfunction, a key early event in atherogenesis, in children with CRF without additional classic risk factors or clinical vascular disease.² One possible mechanism for endothelial damage in CRF is the presence of high circulating levels of homocysteine. Homocysteine is a sulfur-containing amino acid formed as an intermediate during the metabolism of methionine, which has been shown in population studies to be an independent risk factor for both vascular disease^{3,4} and myocardial infarction.^{5,6} In CRF, homocysteine is also an independent risk factor,⁷ and in dialysis patients, hyperhomocystinemia is more prevalent than traditional cardiovascular risk factors.⁸ Homocysteine may, therefore, contribute to aggressive “ac-

celerated atherosclerosis” in CRF. In vitro and in vivo studies suggest that homocysteine causes endothelial dysfunction either directly or via intermediate reactions by increasing oxidized LDL levels.⁹ Even modestly elevated homocysteine levels may be particularly damaging in the presence of the atherogenic risk profile of CRF.¹⁰

Folic acid has been shown to lower homocysteine levels in several populations and can improve brachial artery endothelial function.^{11–13} In CRF, there appears to be relative resistance to folic acid, but supplementation in adults with doses of 5 to 15 mg/day can decrease homocysteine levels by as much as 40% to 50%.¹⁴ The impact on endothelial function has, however, been disappointing.^{15–17}

We report the use of high-resolution ultrasound to study the effect of folic acid supplementation on homocysteine and vascular function in children with moderate to severe CRF. Children were selected specifically both to reduce the influence of confounding factors and, thus, provide a clinical

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model of uremic influences on the arterial wall and to determine whether early intervention might have greater vascular benefits than those seen in adults.

Methods

Subjects

Twenty-five children (11 girls and 14 boys, mean age 12 ± 3 years [range 7 to 17 years]) with CRF (glomerular filtration rate <50 mL/min per 1.73 m^2) were recruited from the outpatient department at Great Ormond Street Hospital for Children. Twenty-four children had congenital structural causes of CRF, and 1 child had an acquired (cortical necrosis) cause of CRF. Sample size was based on an estimated benefit of 2% in flow-mediated dilatation (FMD) with 80% power and a 5% significance level. We excluded children who were smokers, hypertensive, diabetic or nephrotic, or on vasoactive medication or dialysis. No child received folic acid supplementation or vitamins (apart from activated vitamin D) before the study. The local research ethics committee approved the study, and informed consent was obtained from the parents or guardian or from the patient in those >16 years of age.

Study Design

We performed a randomized, placebo-controlled, double-blinded, crossover trial with two 8-week treatment periods separated by an 8-week washout period. Folic acid was given at a dose of 5 mg/ m^2 surface area (Special Products Ltd, Addlestone, Surrey, UK, who also prepared the placebo).

Children were evaluated at the start and the end of each treatment period. At each visit, supine blood pressure was recorded, blood was taken (after a 6-hour fast), and vascular function was assessed.

Assessment of Vascular Function

Endothelial function was determined by recording the dilator response of the brachial artery to increased blood flow generated during reactive hyperemia (FMD). Subjects lay supine in a temperature-controlled laboratory (22°C to 25°C). The brachial artery was scanned in longitudinal section with a 7-MHz linear array transducer and an XP 128/10 (Acuson), magnified using a resolution box function and gated with the R wave of the ECG. End-diastolic images of the vessel were acquired every 3 seconds using data acquisition software (Information Integrity) throughout the whole study and were stored off-line for later analysis. Arterial diameter over a 1- to 2-cm segment was determined for each image using automatic edge detection software (Information Integrity). Analysis was performed by an experienced vascular technician blinded to the phase of the study. With pulse-wave Doppler, blood flow was recorded continuously throughout the study and was expressed as the velocity time integral (area under the blood velocity/time curve for a complete cardiac cycle). Baseline recordings of arterial diameter were made for 1 minute before inflation of a blood pressure cuff placed distal to the site of arterial imaging. Recording continued for 5 minutes during cuff inflation to 300 mm Hg and for 4 minutes after deflation. The time point of maximum change in diameter was also recorded. Endothelium-independent dilatation of the brachial artery was assessed by measuring the dilator response to a 25- μg dose of the nitric oxide (NO) donor, glyceryl trinitrate (GTN) given sublingually. This elicited vascular dilatation of the same magnitude as that of the endothelium-dependent flow stimulus. Results are expressed as both percentage and absolute maximum change in vessel diameter.

Laboratory Assays

Full blood count, urea, creatinine, bicarbonate, and electrolytes were measured (Vitros 750, Ortho-Clinical Diagnostics). Fasting lipid analyses were performed for total cholesterol, HDL, and triglycerides with colorimetric assays (Vitros 750, Ortho-Clinical). LDL values were calculated, and LDL subfractions were measured with high-resolution polyacrylamide gel electrophoresis (Quantimetrix), reported as the ratio of less dense to more dense

TABLE 1. Physical and Biochemical Characteristics at Entry of the 23 Children Who Completed the Study

	Mean \pm SD
Age, years	11.5 ± 3
Sex, male:female	13:10
Height, cm	144.3 ± 17.9
Weight, kg	40.9 ± 14.7
Systolic/diastolic blood pressure, mm Hg	$110 \pm 10/67 \pm 9$
Glomerular filtration rate (NR: 80–120 mL/min per 1.73 m^2)	28.3 ± 12.7
Serum creatinine (NR: 40–102 $\mu\text{mol/L}$)	229 ± 193
Total homocysteine (NR: 4.4–13.7 $\mu\text{mol/L}$)	9.85 ± 3.57
Serum total cholesterol (NR: 3.1–5.4 mmol/L)	4.74 ± 1.05
Serum triglycerides (NR: 0.4–1.4 mmol/L)	1.66 ± 0.65
Hemoglobin (NR: 13–16 g/dL)	12.8 ± 1.49

Normal range (NR) is given where appropriate.

(LDL1+2:LDL3+4+5). LDL lag times were measured by isolating LDL with density-gradient ultracentrifugation and were desalted by gel filtration. Oxidation was promoted with copper, conjugated diene production was monitored, and lag times were generated.¹⁸ Total serum antioxidant activity was measured with a chemiluminescent assay. This is based on a catalyzed oxidation of luminol (chemiluminescent substrate) by hydrogen peroxide, which generates free radicals. The duration of suppression of this reaction by the subject's serum is a measure of its total antioxidant capacity. This is compared against a standard curve created by a calibrant and provides a rapid, reproducible measure of antioxidant defense in biological fluids.¹⁹ Serum and red cell folate levels were determined with a radioimmunoassay (Abbot IMx) with a normal range for serum folate of 2 to 20 $\mu\text{g/L}$ and for red cell folate of 150 to 650 $\mu\text{g/L}$. Plasma total (free and bound) homocysteine was measured with a competitive fluorescence polarization immunoassay (normal range 4.4 to 13.7 $\mu\text{mol/L}$ for adults, Abbot IMx).

Analysis

Each subject served as their own control. The data were tested for normality with the Shapiro-Wilks and the modified Kolmogorov-Smirnov tests. The data were analyzed in 2 ways. First, change in FMD (post-treatment value minus pretreatment value) on folic acid or placebo was compared with a paired *t* test. Second, final FMD after folic acid and after placebo were compared with ANCOVA.²⁰ All descriptive data are expressed as group mean \pm SD, and significance is interpreted as $P < 0.05$.

Results

The clinical and biochemical characteristics of the study group are shown in Table 1. Twenty-three children completed the study. One child was transferred to peritoneal dialysis, and 1 child received a renal transplant.

Effect of Folic Acid

There was no effect of folic acid on hemoglobin or renal function (Table 2). At entry to the study, serum folate ($13.7 \pm 3.58 \mu\text{g/L}$) and red cell folate levels ($334 \pm 202 \mu\text{g/L}$) were normal. Folic acid produced a significant increase in both serum folate (11.7 ± 4.25 to $635 \pm 519 \mu\text{g/L}$, $P = 0.001$) and red cell folate (364 ± 195 to $2891 \pm 2623 \mu\text{g/L}$, $P < 0.001$) levels during the treatment period.

During placebo, there was no change in serum or red cell folate levels when the placebo phase preceded the folic acid

TABLE 2. Biochemical Responses to Folic Acid and Placebo

	Baseline	After Placebo		Baseline	After Folic Acid	
Serum folate (NR: 3–20 $\mu\text{mol/L}$)	17.0 \pm 8.9	12.4 \pm 6.0	NS	13.1 \pm 8.8	635 \pm 519	0.001
Red cell folate (NR: 150–650 $\mu\text{mol/L}$)	596 \pm 468	405 \pm 168	0.02	364 \pm 195	2891 \pm 2623	0.0004
Total homocysteine (NR: 4.4–13.7 $\mu\text{mol/L}$)	9.02 \pm 2.19	9.84 \pm 2.74	NS	10.28 \pm 4.16	8.62 \pm 2.32	0.03
Total antioxidant activity (normal: 440 $\mu\text{trolox Eq}$)	188 \pm 66	216 \pm 74	NS	203 \pm 80	207 \pm 74	NS
LDL lag times (normal: 60 min)	62.8 \pm 17	63.2 \pm 13	NS	58.4 \pm 18	68.4 \pm 25	0.001

Results are given as mean \pm SD. Normal ranges (NR) in brackets. NS indicates not significant.

phase (13.6 \pm 4.6 to 10.68 \pm 5.76 $\mu\text{g/L}$, and 348 \pm 244 to 351 \pm 127 $\mu\text{g/L}$, $P=\text{ns}$). However, in the children who received placebo after folic acid, the serum folate changed from 20 \pm 9.9 to 14.01 \pm 6.08 $\mu\text{g/L}$ ($P=\text{ns}$) and the red cell folate changed from 820 \pm 517 to 470 \pm 185 $\mu\text{g/L}$ ($P=0.02$) during the placebo phase. These postplacebo levels were higher at the end of the study than at entry, which suggested a carry over effect for red cell folate.

Homocysteine Levels

Homocysteine levels at entry to the study were greater (9.85 \pm 3.57 $\mu\text{mol/L}$) than published data on normal children (Table 2). There was a significant fall in total homocysteine levels after folic acid (10.28 \pm 4.16 $\mu\text{mol/L}$ to 8.62 \pm 2.32 $\mu\text{mol/L}$, $P=0.03$) but not in the placebo phase (9.02 \pm 2.19 to 9.84 \pm 2.7 $\mu\text{mol/L}$, $P=0.3$).

Lipid Analysis

Baseline total cholesterol levels were within the normal range (4.74 \pm 1.05 mmol/L), and there was no significant change with treatment or placebo. Triglycerides were elevated above the normal range (1.66 \pm 0.65 mmol/L) and were unchanged after folic acid or placebo (Table 2). HDL and LDL cholesterol were within the normal range at baseline (1.36 \pm 0.36 mmol/L [normal range 0.93 to 1.94] and 2.7 \pm 0.8 mmol/L [normal range 1.63 to 3.63], respectively) and did not change significantly with either treatment or placebo.

Oxidant Stress

Baseline values for LDL lag times were within the normal range (Table 2). There was a significant increase in LDL lag times after folic acid (58.4 \pm 18.7 to 68.1 \pm 25.9 minutes, $P=0.01$) compared with placebo (62.8 \pm 17.4 to 63.2 \pm 13.3 minutes $P=0.92$), which suggests that folic acid supplementation reduced susceptibility of LDL to oxidation. Ratios of LDL to HDL (25 \pm 37 to 24 \pm 34, $P=\text{ns}$) remained unchanged during treatment and placebo phases (22 \pm 32 to 30 \pm 36, $P=\text{ns}$) as did total serum antioxidant activity (204 \pm 80 to 208 \pm 74 on treatment vs 188 \pm 65 to 216 \pm 74 $\mu\text{trolox Eq}$ on placebo, $P=\text{ns}$).

Effect of Folic Acid on Vasomotor Function

There was no significant change in baseline arterial diameter, baseline arterial flow, or peak reactive hyperemia after folic acid or placebo (Table 3).

Endothelial-Dependent Dilatation: FMD

A significant improvement in FMD, expressed as percentage and absolute change in vessel diameter (7.21 \pm 2.81% to

8.47 \pm 3.01%, $P=0.036$, and 0.217 \pm 0.106 cm to 0.252 \pm 0.081 cm, $P=0.47$), was seen after folic acid, which was not seen after placebo (8.20 \pm 3.41% to 8.80 \pm 4.01%, $P=0.44$, and 0.244 \pm 0.102 cm to 0.276 \pm 0.104 cm, $P=0.14$). There was, however, no statistically significant difference in post-treatment FMD after placebo or folic acid ($P=\text{ns}$). Mean time of maximum dilatation after cuff release was not significantly different before or after treatment phases (pre-placebo 54 \pm 16 seconds, pre-folic acid 59 \pm 13 seconds, postplacebo 65 \pm 19 seconds, and post-folic acid 66 \pm 17 seconds). No carry over or period effect on FMD was detected ($P=0.2$ and $P=0.17$, respectively).

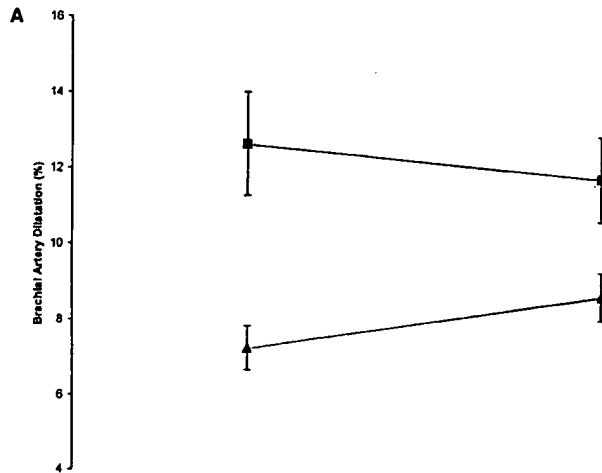
Endothelial-Independent Dilatation: GTN

There was no significant change in response to GTN on either folic acid (12.59 \pm 6.5% to 11.58 \pm 5.39%, $P=0.28$, and 0.374 \pm 0.136 cm to 0.35 \pm 0.129 cm, $P=0.4$) or placebo (12.93 \pm 5.71% to 13.75 \pm 6.46%, $P=0.32$, and 0.390 \pm 0.119 cm to 0.404 \pm 0.170 cm, $P=0.5$). There was no significant change in resting heart rate or supine blood pressure after folic acid or placebo.

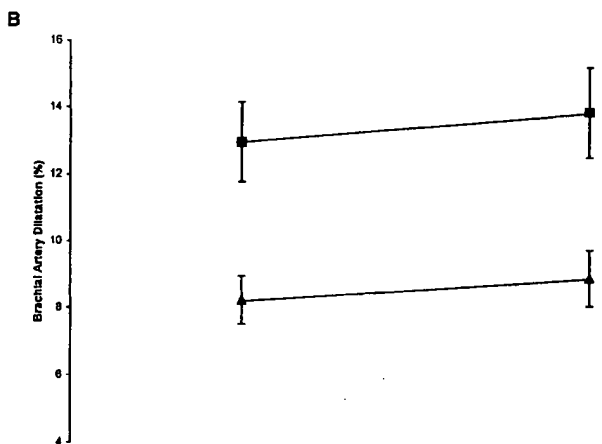
Discussion

This study shows that in children with CRF, supplementation with high-dose folic acid for 8 weeks results in reduction in homocysteine levels, decrease in LDL susceptibility to oxidation, and improvement in endothelial function. These encouraging findings contrast with the disappointing effects of folic acid supplementation on vascular function in adults with renal disease.

Increased cardiovascular mortality and morbidity is well recognized among adults with CRF.²¹ The adverse impact of CRF on cardiovascular mortality and morbidity in the young is, however, even greater, with a 500 \times higher rate of cardiovascular death than a control population.²² Homocysteine levels are consistently elevated in adults with CRF, and this has been suggested to play a role in the pathogenesis of atherosclerosis, especially in view of its strong association with death from vascular disease in the non-uremic population.^{4,6,23} A high prevalence of other risk factors exists in CRF, but an independent association has been found between elevated total homocysteine levels and the risk of myocardial infarction.²⁴ The data on homocysteine in children is limited. Elevated total homocysteine levels (12.6 \pm 5.2 vs 8.2 \pm 3.3 $\mu\text{mol/L}$, $P=0.004$) have been reported in CRF children compared with controls.²⁵ Total homocysteine levels at entry to our study (9.85 \pm 3.57 $\mu\text{mol/L}$) were also elevated in comparison to these controls.



(■ = GTN $p=ns$, ▲ = FMD $p=0.036$).



(■ = GTN $p=ns$, ▲ = FMD $p=ns$).

Change in mean (\pm SEM) brachial artery dilatation on folic acid (A) and placebo (B).

Homocysteine levels can be lowered with folic acid. This increases tissue methylation of homocysteine to form methionine in both uremic and non-uremic individuals, even in the presence of normal folate levels. Studies in adults with hyperhomocystinemia and hypercholesterolemia have shown improvement in endothelial function as a consequence of lowering total homocysteine with folic acid.^{11,13,26} Similar studies in CRF have been disappointing. In patients with CRF and those on dialysis, no improvement in endothelial function has been demonstrated despite significant reductions in homocysteine. Thambyrajah et al¹⁵ recently published a prospective double-blind trial in which 100 adults with a mean glomerular filtration rate of 30 mL/min and a baseline total homocysteine of 20.1 μ mol/L were randomized to either folic acid or placebo. They achieved mean serum folate levels of 39 μ g/L and red cell folate levels of 739 μ mol/L with 5 mg of folic acid for 12 weeks. These values were lower than those achieved in this study. No improvement in endothelial function (using FMD) was seen despite a significant reduc-

tion in total homocysteine. Van Guldener et al²⁷ treated 30 adults on peritoneal dialysis for 12 weeks with 5 mg of folic acid alone or together with 4 g of betaine (an additional co-factor) followed by 1 or 5 mg of folic acid for 40 weeks. Total homocysteine levels were grossly elevated (42.6 μ mol/L) at the beginning of the study and normalized in 40% of patients without any improvement in FMD. In a further attempt to demonstrate long-term clinical benefit from folic acid administration, no improvement in endothelial function was seen after 52 weeks in adult hemodialysis patients, despite a significant reduction in homocysteine levels.¹⁷ Similarly in another population of adults on hemodialysis, carotid artery distensibility and compliance did not change after folic acid supplementation.²⁷ The explanation for these largely negative studies may be due to the particularly aggressive complex nature of the vascular disease, the inability to normalize homocysteine levels in CRF,^{14,28} abnormal folate metabolism, or inadequate folate supplementation.²⁹

We chose to evaluate children because this allowed us to study the process of atherosclerosis early in its natural history, when it is potentially more responsive to intervention. In addition, the young population provided an opportunity to minimize the unquantifiable impact of lifelong confounding risk factors on endothelial function. We excluded children with CRF secondary to inflammatory diseases, diabetes, and hypertension because these are known to have a major impact on vascular function, even in the absence of renal impairment.³⁰ We did not preselect our study population on the basis of FMD or clinical severity of disease so that they would be representative of the effect of CRF in young subjects.

The technique of FMD developed by our group is ideally suited to this study. It is noninvasive, reproducible, and well validated as a measure of NO-dependent vasodilatation and, hence, endothelial function in conduit arteries.³¹ There is good correlation between endothelial-dependent responses in the coronary and forearm circulations.³² The impact of a range of interventions on FMD is well reported both by our group and others in both children and adults with cardiovascular risk factors.

The dose of folic acid in our study produced serum and red cell folate levels higher than in most published clinical intervention studies on CRF patients in the literature, in which endothelial function was the primary endpoint. Variations between 1 mg and 60 mg daily have been used in the renal adult literature with no extra benefit on homocysteine levels conferred by the higher doses. Duration of treatment in adult studies varied from 4 weeks to 52 weeks with the maximum effect on homocysteine seen in the first 2 weeks, and no further lowering occurred despite increasing doses of folic acid.²⁸

At the end of the folic acid treatment period, homocysteine levels had fallen significantly. There was an 8-week washout period between the treatment phases. Analysis of serum and red cell folic acid levels showed that the subjects who received placebo after the active phase had a reduction in red cell folate levels. This implies that there was a "carry over" from the active phase and that ideally the washout period

TABLE 3. Vascular Responses to Folic Acid and Placebo

	Baseline	After Placebo		Baseline	After Folic Acid	
FMD, %	8.2±3.42	8.80±4.01	NS	7.21±2.8	8.49±3.02	0.036
FMD, cm	0.244±0.102	0.276±0.104	NS	0.217±0.106	0.252±0.081	0.047
GTN, %	12.93±5.71	13.75±6.46	NS	12.59±6.53	11.58±5.39	NS
GTN, cm	0.390±0.119	0.404±0.170	NS	0.374±0.136	0.350±0.129	NS
Arterial diameter, mm	3.11±0.57	3.18±0.59	NS	3.13±0.56	3.13±0.58	NS
Resting blood flow (VTI), m	0.058±0.03	0.072±0.03	NS	0.065±0.04	0.074±0.04	NS
Peak reactive hyperaemia, %	680±540	464±233	NS	488±221	494±232	NS

Results are given as mean±SD. NS indicates not significant; VTI, velocity time integral.

could have been longer. There was, however, no carry over effect on homocysteine levels.

There was a significant improvement in FMD during the folic acid treatment phase without change in response to GTN, which suggests a beneficial effect of folic acid on endothelial function after 8 weeks of treatment. It should, however, be noted that the final FMD after placebo and active phases were not significantly different. Our findings must, therefore, be interpreted with caution, and a longer-term trial may be warranted.

The mechanism by which homocysteine exerts its toxic affect on the endothelium is thought principally to be due to the generation of free radical species.⁹ In experimental hyperhomocysteinemia induced by methionine infusion in volunteers, vitamin C improved endothelial function.³³ In our study, we noted a significant reduction in total homocysteine levels with folic acid in parallel with an increase in LDL resistance to oxidation through measurement of lag times. Total antioxidant activity was also measured, but no significant change was noted; thus, increasing the resistance of LDL oxidation might play an important role in the improvement in endothelial function because oxidized LDL is a potent vascular toxin. Alternatively folate may improve endothelial function via endogenous regeneration of tetrahydrobiopterin,³⁴ an essential co-factor in NO production, or through a direct antioxidant effect as shown in vitro.^{34,35}

The improved ability to support renal function in CRF has increased the importance of prevention and treatment of vascular disease. Children are surviving into adult life with prolonged exposure to uremia, and there is good evidence that vascular disease associated with CRF is aggressive and starts very early. Folic acid is safe, lowers homocysteine, reduces LDL susceptibility to oxidation, and may improve endothelial biology relevant to the development of atherosclerosis. Long-term benefits require further study.

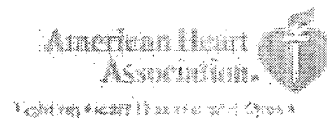
Acknowledgments

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References

- London GM, Drueke TB. Atherosclerosis and arteriosclerosis in chronic renal failure. *Kidney Int.* 1997;51:1678–1695.
- Kari JA, Donald AE, Vallance DT, Bruckdorfer KR, Leone A, Mullen MJ, Bunce T, Dorado B, Deanfield JE, Rees L. Physiology and biochemistry of endothelial function in children with chronic renal failure. *Kidney Int.* 1997;52:468–472.
- Clarke R, Daly L, Robinson K, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. *N Engl J Med.* 1991;324:1149–1155.
- Nygard O, Vollset SE, Refsum H, et al. Total plasma homocysteine and cardiovascular risk profile: the Hordaland Homocysteine Study. *JAMA.* 1995;274:1526–1533.
- Arnesen E, Refsum H, Bona KH, et al. Serum total homocysteine and coronary heart disease. *Int J Epidemiol.* 1995;24:704–709.
- Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. *JAMA.* 1992;268:877–881.
- Moustapha A, Naso A, Nahlawi M, et al. Prospective study of hyperhomocysteinemia as an adverse cardiovascular risk factor in end-stage renal disease [published erratum appears in *Circulation* 1998 Feb 24;97(7):711]. *Circulation.* 1998;97:138–141.
- Bostom AG, Shemin D, Lapane KL, et al. Hyperhomocysteinemia and traditional cardiovascular disease risk factors in end-stage renal disease patients on dialysis: a case-control study. *Atherosclerosis.* 1995;114:93–103.
- Loscalzo J. The oxidant stress of hyperhomocyst(e)inemia. *J Clin Invest.* 1996;98:5–7.
- Baigent C, Burbury K, Wheeler D. Premature cardiovascular disease in chronic renal failure. *Lancet.* 2000;356:147–152.
- Bellamy MF, McDowell IF, Ramsey MW, et al. Oral folate enhances endothelial function in hyperhomocysteinemic subjects [see comments]. *Eur J Clin Invest.* 1999;29:659–662.
- Title LM, Cummings PM, Giddens K, et al. Effect of folic acid and antioxidant vitamins on endothelial dysfunction in patients with coronary artery disease. *J Am Coll Cardiol.* 2000;36:758–765.
- Woo KS, Chook P, Lolin YI, et al. Folic acid improves arterial endothelial function in adults with hyperhomocysteinemia. *J Am Coll Cardiol.* 1999;34:2002–2006.
- Jungers P, Joly D, Massy Z, et al. Sustained reduction of hyperhomocysteinemia with folic acid supplementation in predialysis patients. *Nephrol Dial Transplant.* 1999;14:2903–2906.
- Thambyrajah J, Landray MJ, McGlynn FJ, et al. Does folic acid decrease plasma homocysteine and improve endothelial function in patients with predialysis renal failure? *Circulation.* 2000;102:871–875.
- van Guldener C, Janssen MJ, Lambert J, et al. Folic acid treatment of hyperhomocysteinemia in peritoneal dialysis patients: no change in endothelial function after long-term therapy. *Perit Dial Int.* 1998;18:282–289.
- van Guldener C, Janssen MJ, Lambert J, et al. No change in impaired endothelial function after long-term folic acid therapy of hyperhomocysteinemia in haemodialysis patients. *Nephrol Dial Transplant.* 1998;13:106–112.
- McDowell IF, McEneny J, Trimble ER. A rapid method for measurement of the susceptibility to oxidation of low-density lipoprotein. *Ann Clin Biochem.* 1995;32(pt 2):167–174.
- Chapple IL, Mason GI, Garner I, et al. Enhanced chemiluminescent assay for measuring the total antioxidant capacity of serum, saliva and crevicular fluid. *Ann Clin Biochem.* 1997;34(pt 4):412–421.
- Jones B, Kenward MG. *Design and Analysis of Cross-Over Trials.* London: Chapman and Hall; 1989:60–67.

21. Raine AE, Margreiter R, Brunner FP, et al. Report on management of renal failure in Europe, XXII, 1991. *Nephrol Dial Transplant*. 1992; 7(suppl 2):7-35.
22. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis*. 1998;32: S112-S119.
23. Nygard O, Nordrehaug JE, Refsum H, et al. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med*. 1997;337:230-236.
24. Jungers P, Massy ZA, Khoa TN, et al. Incidence and risk factors of atherosclerotic cardiovascular accidents in predialysis chronic renal failure patients: a prospective study. *Nephrol Dial Transplant*. 1997;12:2597-2602.
25. Lilien M, Duran M, Van Hoeck K, et al. Hyperhomocyst(e)inaemia in children with chronic renal failure. *Nephrol Dial Transplant*. 1999;14:366-368.
26. Verhaar MC, Wever RM, Kastelein JJ, et al. Effects of oral folic acid supplementation on endothelial function in familial hypercholesterolemia: a randomized placebo-controlled trial [see comments]. *Circulation*. 1999;100:335-338.
27. van Guldener C, Lambert J, ter Wee PM, et al. Carotid artery stiffness in patients with end-stage renal disease: no effect of long-term homocysteine-lowering therapy. *Clin Nephrol*. 2000;53:33-41.
28. Sunder-Plassmann G, Fodinger M, Buchmayer H, et al. Effect of high dose folic acid therapy on hyperhomocysteinemia in hemodialysis patients: results of the Vienna multicenter study. *J Am Soc Nephrol*. 2000;11:1106-1116.
29. Massy ZA. Reversal of hyperhomocyst(e)inaemia in chronic renal failure: is folic or folinic acid the answer? *Nephrol Dial Transplant*. 1999;14:2810-2812.
30. Levy D, Kannel WB. Cardiovascular risks: new insights from Framingham. *Am Heart J*. 1988;116:266-272.
31. Sorensen KE, Celermajer DS, Spiegelhalter DJ, et al. Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility. *Br Heart J*. 1995;74:247-253.
32. Selwyn AP, Kinlay S, Ganz P, et al. Atherogenic lipids vascular dysfunction, and clinical signs of ischaemic heart disease. *Circulation*. 1997; 95(1):5-7.
33. Kanani PM, Sinkey CA, Browning RL, et al. Role of oxidant stress in endothelial dysfunction produced by experimental hyperhomocyst(e)inemia in humans. *Circulation*. 1999;100:1161-1168.
34. Loscalzo J. What we know and don't know about L-arginine and NO. *Circulation*. 2000;101:2126-2129.
35. Verhaar MC, Wever RM, Kastelein JJ, et al. 5-Methyltetrahydrofolate, the active form of folic acid, restores endothelial function in familial hypercholesterolemia. *Circulation*. 1998;97:237-241.



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